UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

May 17, 2011

MEMORANDUM

Subject: Efficacy Review for Bath and Tile Disinfecting Cleaner; EPA File Symbol 1677-EGL; DP Barcode: D387107

From: Ibrahim Laniyan, Ph.D.
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5/20/11

To: Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Ecolab Inc.
370 N. Wabasha Street
St. Paul, MN 55102

Formulation from the Label:

Active Ingredient	% by wt.
Sodium Hypochlorite	2.15 %
Other ingredients	97.85 %
Total	100.00 %

Available Chlorine: 2.05%

I. BACKGROUND

The product, Bath and Tile Disinfecting Cleaner (EPA File Symbol 1677-EGL), is a new product. The applicant requested to register the product for use as a disinfectant (sporicide, bactericide, fungicide, virucide), mildewcide, and deodorizer on hard, non-porous surfaces in household, institutional, industrial, commercial, food service, animal care, and hospital or medical environments. The label states that the product is an effective disinfectant in the presence of a 5% serum load. The label states that the product is effective in the presence of 400 ppm hard water when used at a 1:1 dilution to disinfect floors. Studies were conducted at ECOLAB located at the Ecolab Schuman Campus on 655 Lone Oak Drive, in Eagan, MN 55121-1560; and ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated February 8, 2011), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), twelve studies (MRID 483857-09 through 483857-20), Statements of No Data Confidentiality Claims for all twelve studies, and the proposed label.

Note: EPA Form 8570-4 (Confidential Statement of Formula) contains Confidential Business Information. Data or information claimed by the applicant to be FIFRA confidential has not been included in this report.

II. USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: appliances, bathtubs, coolers, countertops, desks, diaper changing tables, diaper pails, drinking fountains, examination tables, floors, playground equipment, shower curtains, showers, sinks, tables, toilets, urinals, and walls. The proposed label indicates that the product may be used on hard, non-porous surfaces including: Corian, enamel, fiberglass, Formica, glass, glazed porcelain, glazed tile, laminate, linoleum, Marlite, metal (e.g., chrome, stainless steel), plastic (polyethylene, polypropylene, polyvinylchloride, vinyl), porcelain, sealed granite, and synthetic marble. Directions on the proposed label provide the following information regarding use of the product:

> As a disinfectant: For heavily soiled areas, a pre-cleaning step is required. Use at full strength. Apply with a wet cloth, mop, brush, scrubber, or by soaking. Allow to remain wet for 5 minutes (10 minutes against *Pseudomonas aeruginosa* and 1 minute against viruses identified on the product label).
>
> As a floor disinfectant against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*: Prepare a use solution by adding 128 ounces of product per 1 gallon of water (a 1:1 dilution). Allow floors to remain wet for 10 minutes.
>
> As a disinfectant against *Clostridium difficile* spores: Apply with a coarse trigger sprayer, cloth, mop, brush, scrubber, or by soaking to thoroughly wet surface. Effective after a 3-minute exposure time. Fecal matter/waste must be thoroughly cleaned from surfaces/objects before disinfection by application with a clean cloth, mop, and/or sponge saturated with the disinfectant product. Cleaning is to include vigorous wiping and/or scrubbing, until all visible soil is removed. Special attention is needed for high-

touch surfaces. Surfaces in patient rooms are to be cleaned in an appropriate manner, such as from right to left, or left to right, on horizontal surfaces, and top to bottom on vertical surfaces, to minimize spreading of the spores. Restrooms are to be cleaned last. Do not reuse soiled cloths.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sporicidal Disinfectant against Clostridium difficile: The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile*. The effectiveness of such a product must be substantiated by data derived from one of the following four test methods: Most recent version (2006) of AOAC Method 966.04 (For the AOAC Method 966.04, testing should be conducted with two separate batches of product, using 30 carriers per batch for testing of registered sterilants; and three separate batches of product (one of which is at least 60 days old), using 60 carriers per batch for testing of hospital disinfectants. For the quantitative tests, the carrier number specified in the test method should be used): AOAC Sporicidal Activity of Disinfectants Test, Method I for *Clostridium sporogenes*; AOAC Method 2008.05: Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface); ASTM E 2414-05: Standard Test Method for Quantitative Sporicidal Three Step Method (TSM) to Determine Efficacy of Liquids, Liquid Sprays, and Vapor or Gases on Contaminated Carrier Surfaces; or ASTM E 2197-02: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile*. Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. The following toxigenic strains of *Clostridium difficile* may be used for testing: ATCC 700792, ATCC 43598, or ATCC 43599. All products must carry a pre-cleaning step, thus no organic soil should be added to the spore inoculum. Results must show a minimum 6 log reduction of viable spores in 10 minutes or less. Control carrier counts must be greater than 10^6 spores/carrier.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for

specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 483857-09 "Bath & Tile Disinfecting Cleaner Quantitative Sporicidal Three-Step Method Against *Clostridium difficile*," by Laurinda Holen. Study conducted at ECOLAB. Study completion date – November 4, 2010. Study Identification Number 1000037.

This study was conducted against spores of *Clostridium difficile* (ATCC 700792). Three lots (Lot Nos. P111792, P020201, and P020202) of the product, Bath & Tile Disinfecting

Cleaner, were tested. The laboratory report referenced Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface-Quantitative Three Step Method First Action 2008 (AOAC Official Method 2008.05) and Standard Test Method for Quantitative Sporicidal Three Step Method (TSM) to Determine Sporicidal Efficacy of Liquids, Liquid Sprays & Vapors or Gases on Contaminated Carrier Surfaces (ASTM 2414-05). Each of the three product lots tested was at least 60 days old at the time of testing. Use solutions were prepared by adding 85.2-90.7 mL of the product and 9.3-t4.8 mL of sterile Milli-Q water (a total of 100.0 mL; calculated to achieve 1.85% available chlorine). Three (3) sterile square glass carriers (5 mm x 5 mm; t mm thick) per product lot were inoculated with 10 µL of a 7-10 day old culture of test organism. The carriers were dried in open Petri dishes in a biosafety cabinet for 64 minutes. The carriers were then dried in closed Petri dishes in a desiccator for at least t2 hours at t5-30°C. Each carrier was transferred to a microcentrifuge tube (Fraction A tube), to which t.5 mL of the use solution had been added. An additional 400 µL of the use solution was added at t minute intervals. The carriers remained exposed to the use solution for 3 minutes at 20±2°C. Following exposure, 600 µL of ice-cold Luria-Bertani (LB) broth with 0.5% sodium thiosulfate was added to each Fraction A tube at 1 minute intervals and agitated. Each carrier was transferred to corresponding microcentrifuge tubes (Fraction B tubes) containing 400 µL sterile water. The remaining disinfectant-inoculum mixture contained in the Fraction A tubes was centrifuged for 6±1 minutes. The resulting pellet was resuspended in 100 µL of sterile water and plated on Brain Heart Infusion Agar with 5% sheep's blood. The Fraction B tubes containing the carriers were sonicated for 5 minutes ± 30 seconds. Following sonication, 600 µL ice-cold LB broth was added to each tube and the tubes were vortexed for ~1 minute. Each carrier was transferred to corresponding microcentrifuge tubes (Fraction C tubes) containing 400 µL ice-cold LB broth. The Fraction B tubes were centrifuged and plated as above. The Fraction C tubes were agitated in an orbital shaker for 30±2 minutes at 35±2°C. Following incubation, the contents were plated on Brain Heart Infusion Agar with 5% sheep's blood. All plates were incubated for 72±4 hours at 35±2°C under anaerobic conditions. Following incubation, the total number of viable spores was calculated for each carrier (three fractions per carrier). Controls included those for carrier counts, purity, sterility, neutralization confirmation, and acid resistance at 2 and 5 minutes.

Note: Protocol deviations/amendments reported in the study were observed.

2. MRID 483857-10 "Bath & Tile Disinfecting Cleaner Hospital Disinfection Efficacy," Test Organisms: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442), by Laurinda Holen. Study conducted at ECOLAB. Study completion date – August 19, 2010. Study Identification Number 1000027.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC t5442). Three lots (Lot Nos. Ptt t792, P020202, and P02040t) of the product, Bath & Tile Disinfecting Cleaner (alternate formulation), were tested according to Ecolab Microbiological Services SOP Method MS003-22 (copy provided) Each of the three product lots tested was at least 60 days old at the time of testing. Use solutions were prepared by adding 586.0-6t3.8 mL of the product and 86.2-t t4.0 mL of sterile Milli-Q water (a total of 700.0 mL; calculated to achieve t.85% available chlorine). Fetal bovine serum was added to each culture to achieve a 5% organic soil load. Sodium stearate was added to each culture to achieve a 0.005% soap residue load. Sixty (60) polished stainless steel penicillin cup carriers per product lot per microorganism were immersed for 15-25 minutes in a 48-54 hour old suspension of test organism, at a ratio of t carrier per 1 mL broth. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was placed in 10 mL of the

use solution for 5 minutes (and also for 10 minutes in testing against *Pseudomonas aeruginosa*) at 20±2°C. [The ECOLAB method protocol suggests that the tubes containing the use solution were swirled after addition of the carriers.] Following exposure, individual carriers were transferred to tubes containing Letheen Broth with 0.5% sodium thiosulfate to neutralize. [The ECOLAB method protocol suggests that tubes containing the neutralizer were shaken thoroughly after addition of the carriers.] All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, purity, sterility, viability (e.g., positive growth), neutralization confirmation, and verification of positive carriers.

Note: Testing conducted on April 23, 2010 against *Pseudomonas aeruginosa* for a 5-minute exposure time showed growth in two subculture tubes for one product lot (i.e., Lot No. P020401). Testing was repeated on May 13, 2010 at a 10-minute exposure time.

Note: The laboratory reported a deviation from EPA GLP standards. No media preparation log sheet was completed for *Pseudomonas* Isolation Agar lot 02/11/10-05-11-10.

Note: Protocol deviations/amendments reported in the study were observed.

3. MRID 483857-11 "Bath & Tile Disinfecting Cleaner Hospital Disinfection Efficacy at a 50% Dilution," Test Organisms: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442), by Laurinda Holen. Study conducted at ECOLAB. Study completion date – October 7, 2010. Study Identification Number 1000038.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. P020201, P020202, and P020302) of the product, Bath & Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS003-22 (copy provided). Each of the three product lots tested was at least 60 days old at the time of testing. Use solutions were prepared by adding 307.42-325.32 g of the product and 374.69-392.58 g of 400 ppm synthetic hard water (a total of 700.00 g; titrated at 380-400 ppm; calculated to achieve 9,574 ppm available chlorine). Use solutions were not tested in the presence of a 5% organic load or soap residue load. Sixty (60) polished stainless steel penicillin cup carriers per product lot per microorganism were immersed for 15-25 minutes in a 48-56 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was placed in 10 mL of the use solution for 10 minutes at 20±2°C. [The ECOLAB method protocol suggests that the tubes containing the use solution were swirled after addition of the carriers.] Following exposure, individual carriers were transferred to tubes containing Letheen Broth with 0.5% sodium thiosulfate to neutralize. [The ECOLAB method protocol suggests that tubes containing the neutralizer were shaken thoroughly after addition of the carriers.] All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, purity, sterility, viability (e.g., positive growth), neutralization confirmation, and verification of positive carriers.

Note: The laboratory reported a failed study set up on July 12, 2010. In the study, carrier counts did not meet the acceptance criterion. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

4. MRID 483857-12 "Bath & Tile Disinfecting Cleaner Supplemental Disinfection Efficacy with *Staphylococcus aureus* - MRSA and *Enterococcus faecalis* - VRE," by Laurinda Holen. Study conducted at ECOLAB. Study completion date – August 12, 2010. Study Identification Number 1000032.

This study was conducted against *Staphylococcus aureus* - MRSA (ATCC 33592) and *Enterococcus faecalis* - VRE (ATCC 51299). Two lots (Lot Nos. P020202 and P02040t) of the product, Bath & Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS003-22 (copy provided). Use solutions were prepared by adding 879.0 mL of the product (Lot No. P020202) and t71.0 mL of sterile Milli-Q water (a total of 1050.0 mL; calculated to achieve 1.85% available chlorine) and by adding 6t3.8 mL of the product (Lot No. P02040t) and 86.2 mL of sterile Milli-Q water (a total of 700.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to each culture to achieve a 5% organic soil load. Sodium stearate was added to each culture to achieve a 0.005% soap residue load. Ten (10) polished stainless steel penicylinder cup carriers per product lot per microorganism were immersed for 15±2 minutes in a 48-56 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was placed in 10 mL of the use solution for 5 minutes at 20±2°C. [The ECOLAB method protocol suggests that the tubes containing the use solution were swirled after addition of the carriers.] Following exposure, individual carriers were transferred to tubes containing Letheen Broth with 0.5% sodium thiosulfate to neutralize. [The ECOLAB method protocol suggests that tubes containing the neutralizer were shaken thoroughly after addition of the carriers.] All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, purity, sterility, viability (e.g., positive growth), neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Staphylococcus aureus* - MRSA (ATCC 33592) was verified on a representative culture. Two individual Mueller Hinton Agar plates were streaked with the prepared culture in three different directions. After streaking, three antibiotic disks were added to each plate. The plates were incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of *Staphylococcus aureus* - MRSA (ATCC 33592) to oxacillin. See page 10 of the laboratory report.

Note: Antibiotic resistance of *Enterococcus faecalis* - VRE (ATCC 51299) was verified on a representative culture. Two individual Mueller Hinton Agar plates were streaked with the prepared culture in three different directions. After streaking, three antibiotic disks were added to each plate. The plates were incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 10.3 mm) confirmed antibiotic resistance of *Enterococcus faecalis* - VRE (ATCC 5t299) to vancomycin. See page t0 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

5. MRID 483857-13 "Bath & Tile Disinfecting Cleaner Supplemental Disinfection Efficacy with *Enterococcus faecium* - VRE," by Laurinda Holen. Study conducted at ECOLAB. Study completion date – August 25, 2010. Study Identification Number 1000044.

This study was conducted against *Enterococcus faecium* - VRE (ATCC 51559). Two lots (Lot Nos. P020202 and P020401) of the product, Bath & Tile Disinfecting Cleaner (alternate formulation), were tested according to Ecolab Microbiological Services SOP Method MS003-22 (copy provided). Use solutions were prepared by adding 385.4 mL of the product (Lot No. P020202) and 64.6 mL of sterile Milli-Q water (a total of 450.0 mL; calculated to achieve 1.85% available chlorine) and by adding 442.6 mL of the product (Lot No. P020401) and 57.4 mL of sterile Milli-Q water (a total of 500.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sodium stearate was added to the culture to achieve a 0.005% soap residue load. Ten (10) polished stainless steel penicylinder cup carriers per product lot were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was placed in 10 mL of the use solution for 5 minutes at 20±2°C. [The ECOLAB method protocol suggests that the tubes containing the use solution were swirled after addition of the carriers.] Following exposure, individual carriers were transferred to tubes containing Letheen Broth with 0.5% sodium thiosulfate to neutralize. [The ECOLAB method protocol suggests that tubes containing the neutralizer were shaken thoroughly after addition of the carriers.] All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, purity, sterility, viability (e.g., positive growth), neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Enterococcus faecium* - VRE (ATCC 51559) was verified on a representative culture. Two individual Mueller Hinton Agar plates were streaked with the prepared culture in three different directions. After streaking, three antibiotic disks were added to each plate. The plates were incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of *Enterococcus faecium* - VRE (ATCC 51559) to vancomycin. See page 10 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

6. MRID 483857-14 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Respiratory syncytial virus," for Bath & Tile Disinfecting Cleaner, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – July 8, 2010. Project Number A09519.

This study was conducted against Respiratory syncytial virus (Strain Long; ATCC VR-26), using Hep-2 cells (human larynx carcinoma cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. P020202-2-CL and P020401-2-CL) of the product, Bath & Tile Disinfecting Cleaner, were tested according to ATS Labs Protocol No. ECO01042810.RSV (copy provided). Use solutions were prepared by adding 293.0-306.9 mL of the product and 43.1-57.0 mL of filter sterilized deionized water (total of 350.0 mL; calculated to achieve 1.85% available chlorine). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Sodium stearate was added to the culture to achieve a 0.005% soap residue load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 1 minute at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium with 2% heat-inactivated

fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, 10 µg/mL vancomycin, 2.0 mM L-glutamine, and 10 mM Hepes. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

7. MRID 483857-15 "Bath & Tile Disinfecting Cleaner Fungicidal Disinfection Efficacy," Test Organisms: *Trichophyton mentagrophytes* (ATCC 9533) and *Aspergillus niger* (ATCC 6275), by Laurinda Holen. Study conducted at ECOLAB. Study completion date – August 19, 2010. Study Identification Number 1000031.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533) and *Aspergillus niger* (ATCC 6275). Two lots (Lot Nos. P020202 and P020401) of the product, Bath & Tile Disinfecting Cleaner (alternate formulation), were tested according to Ecolab Microbiological Services SOP Method MS007-17 (copy provided). Use solutions were prepared by adding 259.3-265.6 mL of the product and 34.4-40.7 mL of sterile Milli-Q water (a total of 300.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to the *Aspergillus niger* culture to achieve a 5% organic soil load. Sodium stearate was added to the *Trichophyton mentagrophytes* and *Aspergillus niger* cultures to achieve a 0.005% soap residue load. Ten (10) polished stainless steel penicylinder cup carriers per product lot per microorganism were immersed for 15±2 minutes in a 7-day old suspension of *Trichophyton mentagrophytes*, or a 13-day old suspension of *Aspergillus niger*, at a ratio of 1 carrier per 1 mL suspension. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was placed in 10 mL of the use solution for 5 minutes at 20±2°C. [The ECOLAB method protocol suggests that the tubes containing the use solution were swirled after addition of the carriers.] Following exposure, individual carriers were transferred to tubes containing Sabouraud Dextrose Broth with 0.5% sodium thiosulfate to neutralize. [The ECOLAB method protocol suggests that tubes containing the neutralizer were shaken thoroughly after addition of the carriers.] All subcultures were incubated for 10 days at 26±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, purity, sterility, viability (e.g., positive growth), and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were observed.

8. MRID 483857-16 "Bath & Tile Disinfecting Cleaner Supplemental Disinfection Efficacy," Test Organisms: *Escherichia coli* (ATCC 11229), *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 7644), *Streptococcus pyogenes* (ATCC 19615), Multiple Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605), *Enterobacter aerogenes* (ATCC 13048), *Streptococcus pneumoniae* (ATCC 6303), and *Shigella dysenteriae* (ATCC 29026), by Laurinda Holen. Study conducted at ECOLAB. Study completion date – November 23, 2010. Study Identification Number 1000034.

This study was conducted against *Escherichia coli* (ATCC 11229), *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 7644), *Streptococcus pyogenes* (ATCC 19615), Multiple Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605), *Enterobacter aerogenes* (ATCC 13048), *Streptococcus pneumoniae* (ATCC 6303), and *Shigella dysenteriae* (ATCC 29026). Two lots (Lot Nos. P020202 and P020401) of the product, Bath &

Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS003-22 (copy provided). Use solutions for testing conducted on May 14, 2010 were prepared by adding 586.0 mL of the product (Lot No. P020202) and 114.0 mL of sterile Milli-Q water (a total of 700.0 mL; calculated to achieve 1.85% available chlorine) and by adding 920.7 mL of the product (Lot No. P020401) and 129.3 mL of sterile Milli-Q water (a total of 1050.0 mL; calculated to achieve 1.85% available chlorine). Use solutions for testing conducted on May 18, 2010 were prepared by adding 879.0 mL of the product (Lot No. P020202) and 171.0 mL of sterile Milli-Q water (a total of 1050.0 mL; calculated to achieve 1.85% available chlorine) and by adding 613.8 mL of the product (Lot No. P020401) and 86.2 mL of sterile Milli-Q water (a total of 700.0 mL; calculated to achieve 1.85% available chlorine). Use solutions for testing conducted on June 28, 2010 were prepared by adding 1037.2 mL of the product (Lot No. P020202) and 162.8 mL of sterile Milli-Q water (a total of 1200.0 mL; calculated to achieve 1.85% available chlorine) and by adding 531.2 mL of the product (Lot No. P020401) and 68.8 mL of sterile Milli-Q water (a total of 600.0 mL; calculated to achieve 1.85% available chlorine). Use solutions for testing conducted on July 15, 2010 were prepared by adding 171.3 mL of the product (Lot No. P020202) and 28.7 mL of sterile Milli-Q water (a total of 200.0 mL; calculated to achieve 1.85% available chlorine) and by adding 177.0 mL of the product (Lot No. P020401) and 23.0 mL of sterile Milli-Q water (a total of 200.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to each culture to achieve a 5% organic soil load. Sodium stearate was added to each culture to achieve a 0.005% soap residue load. Ten (10) polished stainless steel penicylinder cup carriers per product lot per microorganism were immersed for 15±2 minutes (18 minutes for *Enterobacter aerogenes*) in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40±2 minutes at 35±2°C. Each carrier was placed in 10 mL of the use solution for 5 minutes at 20±2°C. [The ECOLAB method protocol suggests that the tubes containing the use solution were swirled after addition of the carriers.] Following exposure, individual carriers inoculated with *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* were transferred to tubes containing Brain Heart Infusion Broth with 0.7 g/L Lecithin and 5.0 g/L Tween 80 to neutralize. All other carriers were transferred to tubes containing Letheen Broth with 0.5% sodium thiosulfate to neutralize. [The ECOLAB method protocol suggests that tubes containing the neutralizer were shaken thoroughly after addition of the carriers.] Subcultures for *Enterobacter aerogenes* were incubated for 48±4 hours at 30±2°C. Subcultures for *Streptococcus pneumoniae* were incubated for 48±4 hours at 35±2°C under 5% CO_2. All other subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, purity, sterility, viability (e.g., positive growth), neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Multiple Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605) was verified on a representative culture. Six individual Mueller Hinton Agar plates (two for each antibiotic tested) were streaked with the prepared culture in three different directions. After streaking, three of the appropriate antibiotic disks were added to each plate. The plates were incubated and, following incubation, the zones of inhibition were measured and documented. The measured zones of inhibition (i.e., 11 mm, 0 mm, and 12.3 mm for gentamicin, ceftazidime, and imipenem, respectively) confirmed antibiotic resistance of Multiple Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605) to gentamicin, ceftazidime, and imipenem. See page 11 of the laboratory report.

Note: The laboratory reported a failed study set up on May 14, 2010. In the study, carrier counts for *Vibrio cholerae* did not meet the acceptance criterion. Positive growth controls were invalid for *Streptococcus pyogenes* and *Listeria monocytogenes*. In addition, neutralization

confirmation controls for *Vibrio cholerae*, *Streptococcus pyogenes*, and *Listeria monocytogenes* did not demonstrate growth in all tubes inoculated. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: The laboratory reported a failed study set up on May 18, 2010. In the study, Multiple Drug Resistant *Acinetobacter baumannii* demonstrated susceptibility to gentamicin. Positive growth controls were invalid for *Streptococcus pneumoniae*. In addition, neutralization confirmation controls for *Streptococcus pneumoniae* and *Escherichia coli* O157:H7 did not demonstrate growth in all tubes inoculated. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: The laboratory reported a failed study set up on June 9, 20 t0. In the study, carrier counts for *Streptococcus pneumoniae* did not meet the acceptance criterion. Positive growth controls were invalid for *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. In addition, neutralization confirmation controls for *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* did not demonstrate growth. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: The laboratory reported a failed study set up on June 28, 2010. In addition, neutralization confirmation controls for *Escherichia coli* O157:H7 did not demonstrate growth in all tubes inoculated. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

9. MRID 483857-17 "Bath & Tile Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Poliovirus," by Lisa Hellickson. Study conducted at ECOLAB. Study completion date – November 22, 2010. Study Identification Number 1000042.

This study was conducted against Poliovirus type 1 (Strain Chat; ATCC VR-1562), using Vero cells (ATCC CCL-81; propagated in-house) as the host system. Two lots (Lot Nos. P020202 and P020401) of the product, Bath & Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS505-04 (copy provided). Use solutions were prepared by adding 13. t mL of the product (Lot No. P020202) and 1.9 mL of sterile Milli-Q water (a total of 15.0 mL; calculated to achieve 1.85% available chlorine) and by adding 88.9 mL of the product (Lot No. P02040t) and 11.1 mL of sterile Milli-Q water (a total of 100.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sodium stearate was added to the culture to achieve a 0.005% soap residue load. Films of virus were prepared by spreading 0.2 mL of virus inoculum evenly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 33-38 minutes at 20.18-2t.17°C at ~50% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 15-30°C. Near the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium, Eagle (EMEM) with Earle's Balanced Salt Solution, Non-Essential Amino Acids, 5% (v/v) heat-inactivated fetal bovine serum, 50-t00 IU penicillin, and 50-100 µg/mL streptomycin. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated for 7-10 days at 35±2°C

in a humidified atmosphere of 5±2% CO_2. The cultures were scored for the presence or absence of unspecified cytopathic effects and cytotoxicity. Controls included those for cell viability, virus stock titer, dried virus count, column titer count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Käber.

Note: The laboratory reported a deviation from EPA GLP standards. Test cell culture was rinsed with phosphate buffered saline that was one week expired on one occasion.

Note: The laboratory reported a failed study set up on June 23, 2010. In the study, a recoverable virus titer of at least 10^4 was not achieved. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: The laboratory reported a failed study set up on August 6, 2010 using Lot No. P020202. In the study, the results of one of the culture wells of the 10^{-1} dilution demonstrated cytopathic effects atypical of Poliovirus type 1. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

10. MRID 483857-18 "Bath & Tile Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A Virus," by Lisa Hellickson. Study conducted at ECOLAB. Study completion date – November 22, 2010. Study Identification Number 1000043.

This study was conducted against Influenza A virus (Strain Hong Kong; ATCC VR-544), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Minnetonka, MN) as the host system. Two lots (Lot Nos. P020202 and P020401) of the product, Bath & Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS505-04 (copy provided). Use solutions were prepared by adding 87.2 mL of the product (Lot No. P020202) and 12.8 mL of sterile Milli-Q water (a total of 100.0 mL; calculated to achieve 1.85% available chlorine) and by adding 88.9 mL of the product (Lot No. P020401) and 11.1 mL of sterile Milli-Q water (a total of 100.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sodium stearate was added to the culture to achieve a 0.005% soap residue load. Films of virus were prepared by spreading 0.2 mL of virus inoculum evenly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20-24 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 15-30°C. Near the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Modified Eagle's Medium with 2% (v/v) heat-inactivated fetal bovine serum, 100 units/mL penicillin, 10 µg/mL gentamicin, 2.5 µg/mL Fungizone, 10 mM Hepes, and SV5 and SV40 antisera. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated for 7-10 days at 35±2°C in a humidified atmosphere of 5±2% CO_2. The cultures were scored for the presence or absence of unspecified cytopathic effects and cytotoxicity. Controls included those for cell viability, virus stock titer, dried virus count, column titer count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Käber.

Note: The laboratory reported a failed study set up on June 22, 2010. In the study, a recoverable virus titer of at least 10^4 was not achieved. The laboratory did not accept the assay.

These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

11. MRID 483857-19 "Bath & Tile Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus Type 37," by Lisa Hellickson. Study conducted at ECOLAB. Study completion date – November 22, 2010. Study Identification Number 1000063.

This study was conducted against Rhinovirus type 37 (Strain 151-1; ATCC VR-1147), using HeLa cells (ATCC CCL-2; propagated in-house) as the host system. Two lots (Lot Nos. P020201 and P020202) of the product, Bath & Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS505-05 (copy provided). Use solutions were prepared by adding 13.1 mL of the product (Lot No. P020201) and 1.9 mL of sterile Milli-Q water (a total of 15.0 mL; calculated to achieve 1.85% available chlorine) and by adding 13.2 mL of the product (Lot No. P020202) and 1.8 mL of sterile Milli-Q water (a total of 15.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sodium stearate was added to the culture to achieve a 0.005% soap residue load. Films of virus were prepared by spreading 0.2 mL of virus inoculum evenly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 56-64 minutes at 20.81°C at 41.79% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 15-30°C. Near the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium, Eagle (EMEM) with Earle's Balanced Salt Solution, Non-Essential Amino Acids, 5% (v/v) heat-inactivated fetal bovine serum, 50-100 IU penicillin, and 50-100 µg/mL streptomycin. HeLa cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated for 7-10 days at 35±2°C in a humidified atmosphere of 5±2% CO_2. The cultures were scored for the presence or absence of unspecified cytopathic effects and cytotoxicity. Controls included those for cell viability, virus stock titer, dried virus count, column titer count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Käber.

Note: Protocol deviations/amendments reported in the study were observed.

12. MRID 483857-20 "Bath & Tile Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes Simplex Virus Type 1," by Lisa Hellickson. Study conducted at ECOLAB. Study completion date – November 22, 2010. Study Identification Number 1000046.

This study was conducted against Herpes simplex virus type 1 (Strain F; ATCC VR-733), using Vero cells (ATCC CCL-81; propagated in-house) as the host system. Two lots (Lot Nos. P020202 and P020401) of the product, Bath & Tile Disinfecting Cleaner, were tested according to Ecolab Microbiological Services SOP Method MS505-04 (copy provided). Use solutions were prepared by adding 13.1 mL of the product (Lot No. P020202) and 1.9 mL of sterile Milli-Q water (a total of 15.0 mL; calculated to achieve 1.85% available chlorine) and by adding 13.3 mL of the product (Lot No. P020401) and 1.7 mL of sterile Milli-Q water (a total of 15.0 mL; calculated to achieve 1.85% available chlorine). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sodium stearate was added to the culture to achieve a 0.005% soap residue load. Films of virus were prepared by spreading 0.2 mL of virus inoculum evenly over

the bottoms of separate sterile glass Petri dishes. The virus films were dried for 33 minutes at 20.66°C at 49.20% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 15-30°C. Near the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium, Eagle (EMEM) with Earle's Balanced Salt Solution, Non-Essential Amino Acids, 5% (v/v) heat-inactivated fetal bovine serum, 50-100 IU penicillin, and 50-100 µg/mL streptomycin. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated for 7-10 days at 35±2°C in a humidified atmosphere of 5±2% CO_2. The cultures were scored for the presence or absence of unspecified cytopathic effects and cytotoxicity. Controls included those for cell viability, virus stock titer, dried virus count, column titer count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Käber.

Note: The laboratory reported a deviation from EPA GLP standards. Test cell culture was rinsed with phosphate buffered saline that was one week expired on one occasion.

Note: The laboratory reported a failed study set up on June 23, 2010. In the study, a recoverable virus titer of at least 10^4 was not achieved. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. See Appendix B of the laboratory report.

Note: Protocol deviations/amendments reported in the study were observed.

V. RESULTS

MRID Number	Organism	Lot No.	Average Log Density Surviving	Log Density of Numbers Control	Log Reduction
			(CFU/ plate)		
483857-09	*Clostridium difficile* spores	P111792	0.28	6.94	6.66
		P020201	0.00	6.94	6.94
		P020202	0.00	6.94	6.94

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ carrier)
		Lot No. P111792	Lot No. P020202	Lot No. P020401	
5-Minute Exposure Time					
483857-10	*Staphylococcus aureus* Test Date: 4/28/2010	1/60	1/60	0/60	2.7×10^6
	Salmonella enterica Test Date: 4/27/2010	0/60	0/60	0/60	2.4×10^6
	Pseudomonas aeruginosa Test Date: 4/23/2010	0/60	0/60	2/60	3.4×10^6
483857-12	*Staphylococcus aureus* - MRSA Test Date: 5/18/2010	---	0/10	0/10	2.1×10^6

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ carrier)
		Lot No. P111792	Lot No. P020202	Lot No. P020401	
483857-12	*Enterococcus faecalis* - VRE Test Date: 5/18/2010	---	0/10	0/10	2.8×10^6
483857-13	*Enterococcus faecium* - VRE Test Date: 7/02/2010	---	0/10	0/10	6.3×10^5
483857-15	*Trichophyton mentagrophytes* Test Date: 6/16/2010	---	0/10	0/10	5.1×10^5
483857-15	*Aspergillus niger* Test Date: 6/16/2010	---	0/10	0/10	1.1×10^6
483857-16	*Escherichia coli* Test Date: 5/14/2010	---	0/10	0/10	2.9×10^6
483857-16	*Shigella dysenteriae* Test Date: 5/14/2010	---	0/10	0/10	$<1.6 \times 10^4$†
483857-16	*Enterobacter aerogenes* Test Date: 5/18/2010	---	0/10	0/10	1.2×10^7
483857-16	*Streptococcus pneumoniae* Test Date: 6/28/2010	---	0/10	0/10	$<1.7 \times 10^4$††
483857-16	Multiple Drug Resistant *Acinetobacter baumannii* Test Date: 6/28/2010	---	0/10	0/10	3.3×10^6
483857-16	*Listeria monocytogenes* Test Date: 6/28/2010	---	0/10	0/10	1.7×10^6
483857-16	*Streptococcus pyogenes* Test Date: 6/28/2010	---	0/10	0/10	4.2×10^6
483857-16	*Escherichia coli* O157:H7 Test Date: 7/15/2010	---	0/10	0/10	7.9×10^6
10-Minute Exposure Time					
483857-10	*Pseudomonas aeruginosa* Test Date: 5/13/2010	0/60	0/60	0/60	4.9×10^6
		Lot No. P020201	**Lot No. P020202**	**Lot No. P020302**	
483857-11	*Staphylococcus aureus* Test Date: 7/9/2010	0/60	0/60	0/60	6.2×10^6
483857-11	*Salmonella enterica* Test Date: 7/21/2010	0/60	0/60	0/60	4.2×10^6
483857-11	*Pseudomonas aeruginosa* Date: 6/30/2010	1/60	0/60	1/60	1.5×10^7

† Reflects average of 4.6×10^3, 1×10^2, and $<1 \times 10^2$ CFU/carrier

†† Reflects average of 1.5×10^3, $<1.0 \times 10^3$, and 2.6×10^3 CFU/carrier

MRID Number	Organism	Results			Dried Virus Count
			Lot No. P020202-2-CL	Lot No. P020401-2-CL	
1-Minute Exposure Time					
483857-14	Respiratory syncytial virus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
			Lot No. P020202	**Lot No. P020401**	
483857-17	Poliovirus type 1 Test Date: 8/06/2010	10^{-1} dilutions	Cytotoxicity (one well)	Cytotoxicity (one well)	
		10^{-2} to 10^{-5} dilutions	---	Complete inactivation	$10^{5.88}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	$\leq 10^{0.5}$	
		Log reduction	≥ 5.25 $\log_{10}$	≥ 5.38 $\log_{10}$	
483857-17	Poliovirus type 1 Test Date: 8/16/2010	10^{-1} to 10^{-5} dilutions	Complete inactivation	---	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	---	
483857-18	Influenza A virus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{4.75}$ $TCID_{50}$/0.1 mL
		10^{-2} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	
483857-20	Herpes simplex virus type 1	10^{-1} dilution	Cytotoxicity (two wells)	Cytotoxicity (one well)	$10^{4.38}$ $TCID_{50}$/0.1 mL
		10^{-2} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log reduction	≥ 3.88 $\log_{10}$	≥ 3.88 $\log_{10}$	
			Lot No. P020202	**Lot No. P020201**	
483857-19	Rhinovirus type 37	10^{-1} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	$10^{4.38}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

VI. CONCLUSIONS

1. The submitted efficacy data (MRID 483857-09) **support** the use of the product, Bath & Tile Disinfecting Cleaner, as a disinfectant against spores of *Clostridium difficil*e on pre-cleaned, hard, non-porous surfaces for a 3-minute contact time. A >6-log reduction in viable spores was reported by the laboratory. At least one of the product lots tested was at least 60 days old at the time of testing. Carrier counts met the acceptance criterion of >1.0 x 10^6 spores/carrier. Neutralization confirmation testing demonstrated that the neutralizer was not sporicidal and was effective in neutralizing the product. Purity controls were reported as pure. Sterility controls did not show growth. Test spores showed resistance to acid for ≥5 minutes.

2. The submitted efficacy data **support** the use of the product, Bath & Tile Disinfecting Cleaner, as a disinfectant with bactericidal/ fungicidal activity against the following microorganisms on

hard, non-porous surfaces in the presence of a 5% organic soil load and 0.005% soap residue for a 5-minute contact time (for a 10-minute contact time against *Pseudomonas aeruginosa*):

Salmonella enterica	MRID 483857-10
Staphylococcus aureus	MRID 483857-10
Pseudomonas aeruginosa	MRID 483857-10
Staphylococcus aureus - MRSA	MRID 483857-12
Enterococcus faecalis - VRE	MRID 483857-12
Enterococcus faecium - VRE	MRID 483857-13
Aspergillus niger	MRID 483857-15
Escherichia coli	MRID 483857-16
Enterobacter aerogenes	MRID 483857-16
~~Multiple Drug Resistant~~ *Acinetobacter baumannii*	MRID 483857-16
Listeria monocytogenes	MRID 483857-16
Streptococcus pyogenes	MRID 483857-16
Escherichia coli O157:H7	MRID 483857-16

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. [Note that repeat testing was conducted on one product lot against *Pseudomonas aeruginosa* to evaluate a greater contact time.] In testing against *Salmonella enterica*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth. Positive carriers were shown to be positive for growth of the challenge microorganisms. The Master label must reflect the antibiotics for which resistance has been demonstrated in order to support multi-drug resistant claim.

3. The submitted efficacy data **do not support** the use of the product, Bath & Tile Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load and 0.005% soap residue for a 5-minute contact time:

Shigella dysenteriae	MRID 483857-16
Streptococcus pneumoniae	MRID 483857-16

Although complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots, carrier counts did not meet the acceptance criterion. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

4. The submitted efficacy data (MRID 483857-15) **support** the use of the product, Bath & Tile Disinfecting Cleaner, as a disinfectant with fungicidal activity against *Trichophyton mentagrophytes* on pre-cleaned, hard, non-porous surfaces in the presence of 0.005% soap residue for a 5-minute contact time.

5. The submitted efficacy data **support** the use of the product, Bath & Tile Disinfecting Cleaner, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous

surfaces in the presence of a 5% organic soil load and 0.005% soap residue for a 1-minute contact time:

Respiratory syncytial virus	MRID 483857-14
Poliovirus type 1	MRID 483857-17
Influenza A virus	MRID 483857-18
Rhinovirus type 37	MRID 483857-19
Herpes simplex virus type 1	MRID 483857-20

Recoverable virus titers of at least 10^4 were achieved. In studies against Poliovirus type 1, Influenza A virus, and Herpes simplex virus type 1, cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against all other viruses, cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

6. The submitted efficacy data **support** the use of a 9,574 ppm available chlorine dilution (a 1:1 dilution) of the product, Bath & Tile Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganisms on pre-cleaned, hard, non-porous surfaces in the presence of 400 ppm hard water for a 10-minute contact time:

Salmonella enterica	MRID 483857-11
Staphylococcus aureus	MRID 483857-11
Pseudomonas aeruginosa	MRID 483857-11

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth. Positive carriers were shown to be positive for growth of the challenge microorganisms.

VII. LABEL

1. The proposed label claims that the product, Bath and Tile Disinfecting Cleaner, is an effective disinfectant against *Clostridium difficile* spores on pre-cleaned, hard, non-porous surfaces for a 3-minute contact time. **This claim is acceptable as it is supported by the submitted data.**

2. The proposed label claims that the product, Bath and Tile Disinfecting Cleaner, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum and 0.005% sodium stearate (soap scum) for a 5-minute contact time (for a 10-minute contact time against *Pseudomonas aeruginosa*):

Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus

Acinetobacter baumannii (~~MDR~~)
Enterobacter aerogenes
Enterococcus faecalis (VRE)

Enterococcus faecium (VRE)
Escherichia coli
Escherichia coli O157:H7
Listeria monocytogenes
Staphylococcus aureus (MRSA)
Streptococcus pyogenes

Aspergillus niger

These claims are acceptable as they are supported by the submitted data. The Master label must reflect the antibiotics for which resistance has been demonstrated in order to support Multi-Drug Resistant claim.

3. The proposed label claims that the product, Bath and Tile Disinfecting Cleaner, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum and 0.005% sodium stearate (soap scum) for a 5-minute contact time:

Shigella dysenteriae
Streptococcus pneumoniae

Data provided in the data package **do not support** this claim. As noted in the "Conclusions" section of this report, carrier counts did not meet the acceptance criterion. **Efficacy data fully meeting Agency standards must be provided to support disinfectant claims for *Streptococcus pneumoniae* and *Shigella dysenteriae*.**

4. The proposed label claims that the product, Bath and Tile Disinfecting Cleaner, is an effective disinfectant against *Trichophyton mentagrophytes* on hard, non-porous surfaces in the presence of 0.005% sodium stearate (soap scum) for a 5-minute contact time. **This claim is acceptable as it is supported by the submitted data. The directions for disinfecting against *Trichophyton mentagrophytes* must be revised to clearly specify a pre-cleaning step as efficacy was not demonstrated in the presence of a 5% organic soil load.**

5. The proposed label claims that the product, Bath and Tile Disinfecting Cleaner, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 5% blood serum and 0.005% sodium stearate (soap scum) for a 1-minute contact time:

Herpes simplex virus type 1
Influenza A virus
Poliovirus virus type 1
Respiratory syncytial virus
Rhinovirus type 37

These claims are acceptable as they are supported by the submitted data.

6. The proposed label claims that a 1:1 dilution of the product, Bath and Tile Disinfecting Cleaner, is an effective disinfectant against the following microorganisms on floors in the presence of 400 ppm hard water for a 10-minute contact time:

Staphylococcus aureus
Salmonella enterica
Pseudomonas aeruginosa

These claims are acceptable as they are supported by the submitted data. The directions for this application must be revised to include the following (or a similar) statement as efficacy was not demonstrated in the presence of a 5% organic soil load: "Pre-clean floors prior to disinfecting with a 1:1 dilution of the product."

7. The proposed label states that the product can be used as a deodorizer. **The label must be revised to provide adequate dosage recommendations and complete directions for use of the product as a deodorizer.**

8. Marketing claims on pages 6 and 7 of the proposed label state that the product sanitizes surfaces. **Data were not provided to support these claims. References to use of the product as a sanitizer must be deleted from the proposed label.**

9. The applicant must make the following changes to the proposed label, as appropriate:

- On page 2 and 7 of the proposed label, qualify claims regarding product effectiveness as a "one-step" disinfectant. **Surfaces must be pre-cleaned when the product is used to treat against *Clostridium difficile* and *Trichophyton mentagrophytes*, and as a floor disinfectant.**

- On page 4 of the proposed label, change "***Enteroboacter*** *aerogenes*" to read "***Enterobacter*** *aerogenes*."

- On page 9 of the proposed label, change "Enamel" to read "**Baked** enamel." Enamel is a porous surface.

- On page 9 of the proposed label, change "Fiberglass" to read "**Sealed** fiberglass." Fiberglass is a porous surface.

- On page 9 of the proposed label, change "Porcelain" to read "**Glazed** porcelain." Porcelain is a porous surface.